

**CREEK CHUB AS A SENTINEL INDICATOR SPECIES
FOR RELATIVE REGIONAL ASSESSMENT OF
MERCURY CONTAMINATION AND MONITORING
POLLUTANT TRENDS IN INDIANA RIVERS AND
STREAMS**

**Biological Studies Section
Assessment Branch
Office of Water Quality
Indiana Department of Environmental Management
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James R. Stahl and Stacey L. Sobat

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Executive Summary

The Indiana Department of Environmental Management (IDEM) collects fish tissue to assess environmental contaminants and their ability to bioaccumulate. At present, tissue contaminant monitoring provides data necessary for issuing fish consumption advisories. The Biological Studies Section implemented fish tissue contaminant monitoring into the stratified probabilistic site selection design of the Watershed Monitoring Program in 1997 (Stahl 1997a). The purpose of collecting fish tissue samples using a stratified probabilistic site selection design was to enable IDEM environmental scientists to measure the regional magnitude and extent of fish tissue contamination by specific bioaccumulating elements or compounds, the associations of pollutants with predominant land use(s), and develop indicators for trend analysis of contaminant concentrations in watersheds over time.

The purpose of this report is to evaluate the first two years of mercury analytical results in creek chub whole fish tissue samples collected from probabilistic sites to estimate the proportion of creek chub exceeding a critical value for mercury risk to wildlife. The creek chub tissue samples were collected from probabilistic sites in three watershed basins: East Fork White River (EFWR) 1997, Whitewater River (WWR) 1997, and Upper Wabash River 1998. Creek chub was proposed to be the primary sentinel species when evaluating the bioaccumulation of contaminants because creek chub are ubiquitous (Gerking 1945), show the ability to bioconcentrate contaminants, and are preyed upon by wildlife. Creek chub has also been the highest priority fish species taken for fish tissue at probabilistic and targeted sites for all years in which fish tissue was taken. Mercury was the chosen pollutant to evaluate in creek chub tissue samples because mercury is detected ubiquitously in fish tissue samples from Indiana waters, is a human health concern for protection of individuals from exposure to toxic elements, and is listed as an agency wide priority for reduction in Indiana's environment.

Twelve whole creek chub samples between 10 and 18 cm were collected from the EFWR Basin (1997), 10 samples from the WWR Basin (1997), and 13 from the Upper Wabash River Basin in 1998. Twenty-five whole creek chub samples between 10 and 18 cm were part of the statewide historical target sites database. Mean mercury concentrations in creek chub tissue collected from fish community sites for all watershed basins combined (23.3 parts per billion [ppb]) was significantly different ($p \leq 0.05$) from mercury

concentrations in whole creek chub tissue taken from historical target sites (64.4 ppb); thus, by targeting sites, bias is introduced into the overall estimate of contaminant levels for the region.

There was no significant difference ($p \leq 0.05$) in the log 10 mean mercury concentration of creek chub tissue samples between the EFWR Basin (1.2835 ppb), WWR Basin (1.4595 ppb), and Upper Wabash River Basin (1.3720 ppb). Since these three distinct watersheds have no significant difference among log 10 mean mercury concentrations, a hypothesis can be made and tested to see if atmospheric mercury deposition is the most influential source of mercury to the aquatic environment. To make an estimation of the mean mercury level in creek chub tissue with a 90% level of confidence, the minimum sample size needed was 13 creek chub tissue samples, based on pooled 97/98 probabilistic creek chub tissue samples ($n=35$). By successfully collecting 35 creek chub tissue samples from probabilistic fish community sites, a confidence level of 94% was attained that the mean mercury concentration for all watersheds combined is 23.3 ppb in whole creek chub tissue samples.

To make regional inferences using creek chub, it is important to understand the distribution of tissue samples in relation to the original stratification design. The proportion of fish community sites sampled using a stratified probabilistic site selection design did not deviate significantly ($p \leq 0.05$) from the expected equal proportions of stream orders for the basin. This means that all fish community samples collected for the basin equally represent all stream orders so that the biotic integrity of the region can be assessed for all stream miles without bias. All creek chub tissue samples (10-18 cm) taken from fish community assessment sites were collected from streams of third order or less; thus, the creek chub tissue samples are not representative of the entire watershed basin. Inferences based on the levels of mercury in creek chub would apply, therefore, to the smaller order streams. However, these smaller order streams make up as much as 90% of the stream miles in our three watersheds sampled (U.S. EPA NHEERL, Western Ecology Division). With this information on the cumulative distribution frequency of mercury levels in creek chub from the three watersheds, inferences can be made that as much as 40% of creek chub from first, second, and third order streams in the three watersheds may have mercury levels that could pose a risk to avian fish eating wildlife and 15% to mammalian predators of fish.

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INTRODUCTION

The Indiana Department of Environmental Management's (IDEM) fish tissue contaminant monitoring program has become an important and highly profiled program in the State of Indiana. It is a widely used method of monitoring and assessing environmental contaminants and their bioavailability. Concentrations of some contaminants may be greater in tissues than in surrounding waters because of bioaccumulation and biomagnification. Tissue contaminant monitoring provides a tool that measures contaminants in Indiana's environment that may not be detected in water or air. It also provides information on the availability of compounds that bioaccumulate. Tissue contaminant monitoring, when part of an integrated multimedia monitoring program, gives insight into exposure levels and will enable IDEM to better develop its understanding of the complexities of contaminant distribution, fate, and effects (IDEM 1998a).

The Assessment Branch in the Office of Water Quality has implemented the management framework set forth in the revised Surface Water Quality Monitoring Strategy and its associated time line in order to establish a scientifically defensible foundation of information from which management decisions can be made (IDEM 1998b). To this end, the Biological Studies Section is developing and assessing ecological indicator methods for aquatic communities and bioaccumulating contaminants. The implementation of the revised Surface Water Quality Monitoring Strategy supports the biennial 305(b) Water Quality Report (IDEM 1996a), National Pollutant Discharge Elimination System (NPDES) Permitting Program, the Indiana Fish Consumption Advisory (FCA) (ISDH 1999), and the 1999-2001 Environmental Performance Partnership Agreement (EnPPA) (IDEM 1999).

Because of the historical nature of the fish tissue contaminant monitoring program (i.e. to support the issuance of fish consumption advisories for the protection of human

health, and to locate stream reaches/lake acres with contamination), IDEM staff have not been able to adequately estimate regional risks of consumption to predators nor to objectively track how the risk changes through time. To make objective estimates of regional contaminant levels in fish tissue, a program needs to be established that has a sampling design from which all stream reaches have an equal probability of being sampled. Coupling fish contaminant monitoring with a probabilistic (random) site selection approach for sampling can enable environmental scientists to make reasonable relative estimates of contaminant levels, based on sentinel species, for entire watersheds and form trend lines over time (Paulsen *et al.* 1993; Yeardley 1994; Yeardley *et al.* 1996).

The need to better understand the condition of ecological resources with regard to contaminant bioaccumulation at a more regional scale prompted the implementation of contaminant monitoring of fish tissue at the probabilistic design sites of the Watershed Monitoring Program in 1997 (IDEM 1998c). This randomized site selection monitoring design was created to answer a number of questions such as the magnitude and extent of contamination, the associations of contaminants with predominant land use(s), responses to changes over time (trends), spatial extent, and hot spot determinations concerning contaminant fate and transport. Unbiased representation of the resource is the most critical component in the process of establishing reliable estimates of regional conditions (Peterson *et al.* 1999). This, combined with other sampling efforts at the same locations (i.e. fish community, surface water chemistry, benthic aquatic macroinvertebrate community, and qualitative habitat assessments), will provide a more holistic view of the resource as a step toward ecological integrity assessment (IDEM 1998c, 1998d, 1998e). Information based on a probabilistic design approach to monitoring can be used by regional resource managers, legislators, and the public to prioritize environmental concerns and decide what types of remediations, abatements, best management practices, and/or legislative efforts may be needed to improve and protect these resources.

The purpose of this report is to evaluate the first two years of mercury analytical results in creek chub whole fish tissue samples collected from probabilistic sites in the East Fork White River (EFWR) Basin (1997), the Whitewater River (WWR) Basin (1997), and upper Wabash River Basin (1998) to estimate the proportion of creek chub exceeding a critical value for mercury risk to wildlife. Several questions that need to be answered include: 1) Is there a difference in mercury levels in whole creek chub tissue from probabilistic sites versus historical target sites?, 2) Are there differences in levels of mercury in whole creek chub tissue between the sampled basins?, 3) Given that collecting whole creek chub fish tissue samples at the probabilistic sites is not going to be one hundred percent successful, what is the estimated minimum number of samples necessary to attain a desired confidence in the estimation of the mean mercury level in creek chub tissue?, 4) Is the program attaining the desired success for the collection of this primary target species to warrant the continuation of this sampling effort?, and 5) What are the evident limitations in making regional inferences using creek chub as a sentinel species?

Creek Chub As A Sentinel Indicator Species for Estimating Regional Conditions

There are a number of reasons why creek chub (*Semotilus atromaculatus*) is proposed to be the primary sentinel or “canary” species in looking at bioaccumulation of contaminants in fish tissue at a regional scale. First, the creek chub is one of the most widely distributed and available species of minnows in Midwestern North America (Simon, per comm.). They are abundant in small headwater creeks, as well as, medium and large size streams. In Indiana, creek chub are found almost ubiquitously (except in lakes) (Gerking 1945). This makes it likely that an adequate sample of creek chub will be obtainable from the sites that are selected using a stratified probabilistic design. In addition, creek chub have very small home ranges (Pflieger 1997). The species is tolerant and can even be found surviving in isolated stream ponds during low flow periods. The creek chub is a generalized carnivore, adapting to whatever diet is available (Pflieger 1997). Smaller individuals will feed mainly on benthic macroinvertebrates while large individuals will feed on other minnows, crayfish, mollusks, and worms. Creek chub are preyed upon by a number of wildlife including heron, kingfisher, loons, and merganser. As river otters become reestablished in the State of Indiana, creek chub could become an important prey item in their diets as well.

The statewide average percent fat content for whole creek chub (IDEM fish tissue contaminant database) is 3.65 (geometric mean based on natural log is 3.08 percent). Many lipophilic organochlorine compounds such as PCBs, chlordane, DDT, and dieldrin have been detected in creek chub tissue samples (IDEM fish tissue contaminant database). Mercury is also commonly detected in creek chub tissue. This species has traditionally been collected at targeted problem sites on small streams. At some of these locations, creek chub is the key species in assigning a FCA. The ubiquity of creek chub, the demonstrated ability to bioconcentrate organochlorine contaminants and mercury, and its potential use by fish eating wildlife make creek chub an ideal test species for implementation of a fish tissue contaminant monitoring program based on a stratified probabilistic site selection design in Indiana.

Mercury

Mercury can exist in a number of forms in the environment. In the atmosphere, mercury occurs as an elemental vapor, which circulates in the atmosphere for up to a year, and can therefore widely disperse and transport thousands of miles (U.S. EPA 1997b). Mercury has found widespread use in insecticides, fungicides, bactericides, pharmaceuticals, paint additives, leather tanning, batteries, electrical equipment, applications in metallurgy, dental fillings, thermometers, and barometers. It is also released from fossil fuel combustion. Use of mercury in pesticides is now banned except for some limited use in fungicides or preservatives (International Joint Commission (IJC) 1991). Natural emissions of mercury include release from soils, vegetation, forest fires, and water surfaces (IJC 1991).

Mercury, mobilized and released into the environment, has increased since the beginning of the industrial age (U.S. EPA 1997a). Mercury released in this century through human activities is almost ten times the calculated amount released due to natural weathering (Moore and Ramamoorthy 1984). Recent estimates place the annual amounts of mercury released into the air by human activities at between 50 and 75% of the total yearly input to the atmosphere from all sources (U.S. EPA 1997b). However, the IJC (1991) estimated man-made emissions of mercury to be less than half the natural mercury emissions. Slemr and Langer (1992) estimated that atmospheric concentrations of total gaseous mercury in the Northern Hemisphere increased about 1.5% per year during 1977 through 1990, which they attributed to anthropogenic releases. However, industrial consumption of mercury has declined by about 75% between 1988 and 1996 due mainly to the elimination of mercury in paints and reduced use in batteries (U.S. EPA 1997b). Total human contribution of mercury into the environment in the United States has been estimated to be 650 metric tons (U.S. EPA 1986b; IJC 1991) with the most important sector being the combustion of fossil fuels.

The use and disposal of consumer products containing mercury releases more mercury (tons/year) to the overall environment than do manufacturing processes (U.S. EPA 1986b; IJC 1991). About 60% of mercury used in the United States go to landfills (IJC 1991). Current relative contributions and levels of mercury to the Indiana environment are not known at this time. It is also not certain whether overall atmospheric mercury levels are currently increasing, decreasing or remaining stable. Some remote site studies suggest that the global atmospheric burden of mercury is still increasing (U.S. EPA 1997a).

Mercury accumulates most efficiently in the aquatic food web (U.S. EPA 1997c). It is detected ubiquitously in fish tissue samples from Indiana waters. Mercury has been detected in 95% of all fish tissue samples analyzed since 1983 (IDEM, Office of Water Quality, Fish Tissue Contaminant Database). Nearly all of the mercury that accumulates in fish tissue is methylmercury (U.S. EPA 1997d). Fish consumption dominates the pathway for human and wildlife exposure to methylmercury (U.S. EPA 1997d). The levels of mercury found in fish tissue and the concern for human health protection from exposure to mercury has prompted the inclusion of a risk-based approach for contaminated fish in the Indiana Fish Consumption Advisory. An assessment of mercury data in common carp, largemouth bass, and channel catfish tissues from the West Fork White River Basin have indicated that mercury concentrations in fish tissue have not changed when comparing the 1980's to the 1990's (Stahl 1997b). The indication was that levels of mercury in fish tissue are staying about the same in the West Fork White River Basin and may be higher than a statewide average.

MATERIALS AND METHODS

Study Area

Sites were randomly selected in the target basins based on a stratified probabilistic design to give equal weight probability of any given stream reach being selected (U.S.

EPA, Environmental Research Laboratory, Corvallis, OR). Streams in the target watersheds were proportioned to stream order (Strahler 1957) based on the one in one hundred thousand scale of U.S. EPA's River Reach File 3. The proportions were weighted to give equal probability of selection to four categories: first order, second order, third order, and fourth and greater orders. The 1997 watershed monitoring focused on the East Fork of the White River Basin (USGS hydrologic cataloging units 05120204 through 05120208), and the Whitewater River Basin (USGS hydrologic accounting unit 050800). Each of these basins had a separate site draw. The 1998 watershed monitoring focused on the upper Wabash River Basin including the Tippecanoe River watershed (USGS hydrologic cataloging units 05120101 through 05120107).

Sample Collection

Fish tissue samples were selected, prepared, and preserved on dry ice in the field after electrofishing activities for fish community assessment were completed and voucher specimens preserved. Preparation of tissue samples entailed measuring each individual fish for length and weight, fillet or wrap whole fish in aluminum foil, and placing in a labeled zip lock or other food grade plastic bag. In all years of this sampling effort to date, creek chub has been the highest priority species for tissue collection in the watershed monitoring program (Stahl 1997a; Stahl 1998; Stahl 1999). It is the first "primary target species" of a prioritized list of fish species for collecting fish tissue from a site. After 1997, if creek chub were not present the investigators were to go to the next species on the list. This helped assure that some kind of primary target fish species was collected at as many sites as possible. Fish were composited together so that there was enough tissue in the sample to meet laboratory minimum sample size requirements. A total composite sample weight of 100 to 400 grams was desired.

The target size class for whole creek chub was 10.0 to 18.0 centimeter (cm) total mean length for composite tissue sample (Stahl 1997a). This size class was considered to be a two to four years age class (Scott and Crossman 1975). The smallest and largest individuals of a composited sample were to be within 90% of each other for total length (Anderson *et al.* 1993). In the second year of this study, the size difference between the smallest and largest individual was expanded so that as low as 75% would be tolerated in order to obtain an adequate composite sample if one would otherwise not be obtainable (Stahl 1998). The total length (cm) of each individual in the sample was recorded on a field sheet so that length range and mean length could be determined. The total sample weight (grams) was recorded by weighing all fish in the sample together before packaging so that an average individual fish weight could be calculated. The weights for the largest and the smallest individuals were recorded to obtain a weight range.

For all aspects of this project, the Biological Studies Section Standard Operating Procedures on fish tissue sample preparation were followed (IDEM 1993). Each composite sample of whole creek chub tissue was wrapped in aluminum foil and labeled. This package was then placed in a food grade zip lock type bag and again labeled. Samples were then placed on dry ice after returning to the vehicle and were kept frozen until they could be placed in the laboratory freezer for storage. For day trips, samples were placed on wet ice until staff could return to the lab at the end of the day.

Laboratory Analyses

Samples were shipped frozen by priority overnight express to the contract analytical laboratory. Analyses on all probabilistic site samples and most target site samples were performed by En Chem, Inc. at their Madison, Wisconsin laboratory using EPA test method SW846 7471A (U.S. EPA 1986a) in strict adherence to the technical specifications of the Broad Agency Announcement BAA 95-34 (IDEM 1995), and the Quality Assurance Project Plan for Indiana Surface Water Programs (IDEM 1996b). The Indiana State Department of Health Food and Dairy laboratory had analyzed a small percentage (2 samples) of the targeted site creek chub tissue samples.

Statistics

All statistical and graphical analyses were performed with STATISTICA 5.1 software. Only creek chub samples analyzed as whole fish and between 10.0 and 18.0 cm were included in the assessments and statistical comparisons. Data sets of creek chub tissue mercury results from probabilistic sampling sites in the East Fork White River watershed (EFWR) 1997, the Whitewater River watershed (WWR) 1997, the upper Wabash River watershed 1998 including the Tippecanoe River Basin, 1997 pooled, 1998, 97 and 98 pooled, and a historical set of targeted sampling sites across Indiana were compared. Mercury results that were less than the detection limit were censored to half the detection limit. Regional contaminant assessment should not be seriously affected by this estimation of concentrations that fell below detection limits.

RESULTS

Twelve whole creek chub samples between 10 and 18 cm were collected from the EFWR Basin (1997), 10 samples from the WWR Basin (1997), and 13 from the upper Wabash River Basin in 1998 (including the Tippecanoe River Basin). Twenty-five whole creek chub samples between 10 and 18 cm were queried from the historical target sites database. The stratified probabilistic draw for the fish community assessment sites was based on an equal weight proportioning on stream order (i.e. first, second, third, and fourth and greater). Figures 1 and 2 show the distribution of the probabilistic fish community sampling sites to their drainage areas and to their stream orders (Strahler 1957) respectively. This represents the potential for 100% success in collecting creek chub tissue samples. For all of the basins, the proportions of sites decrease with increasing drainage area. However, the proportion of sites within each of the defined stream order category tends to follow the stratified design of equal proportion weighting for each of the stream order categories.

For all three of the basins, the proportion of creek chub tissue samples collected decreases with increasing drainage area (Figure 3). One hundred percent of the creek chub tissue samples came from stream segments of less than 100 square miles drainage area for the WWR and the EFWR, or third order streams and lower (Figure 4). Ninety three percent of the upper Wabash River Basin creek chub tissue samples came from sites of less than 100 square miles drainage area, or third order streams and lower.

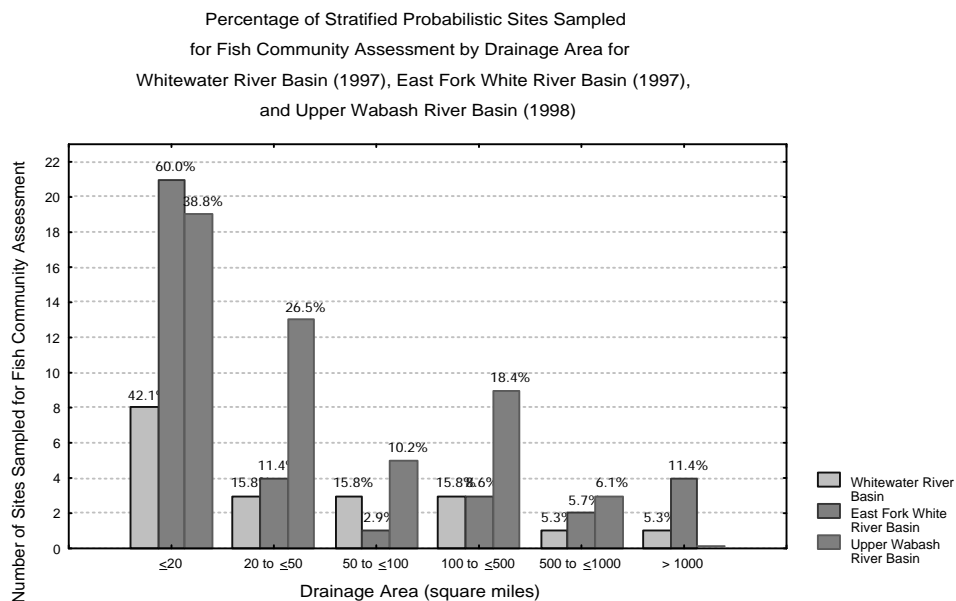


Figure 1. The proportion of stratified probabilistic sites sampled for fish community assessment (potential for creek chub tissue sample collection success) by watershed drainage area for the Whitewater River Basin (1997), East Fork White River Basin (1997), and Upper Wabash River Basin (1998).

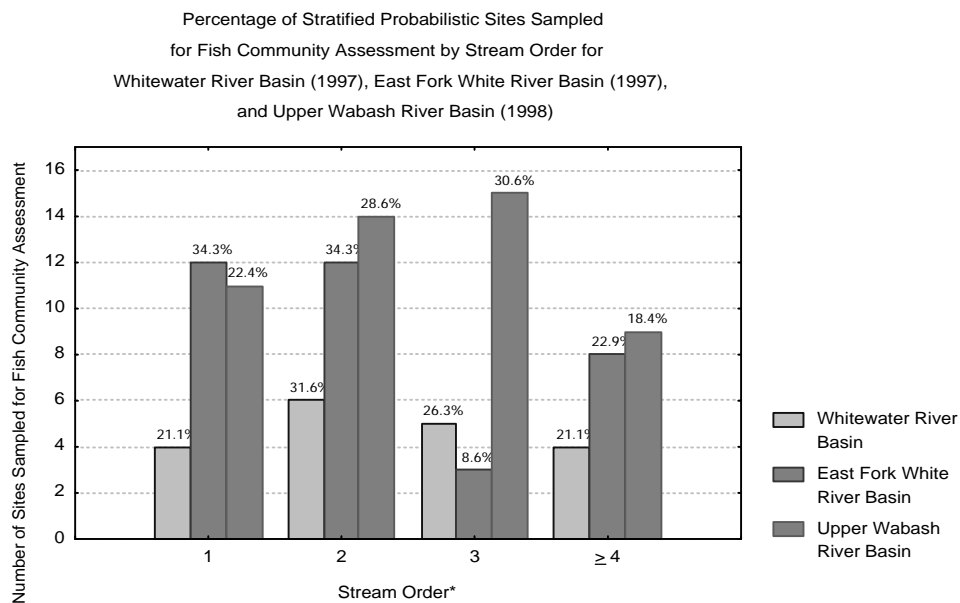


Figure 2. The proportion of stratified probabilistic sites sampled for fish community assessment (potential for creek chub tissue sample collection success) by stream order (Strahler 1957) for the Whitewater River Basin (1997), East Fork White River Basin (1997), and Upper Wabash River Basin (1998).

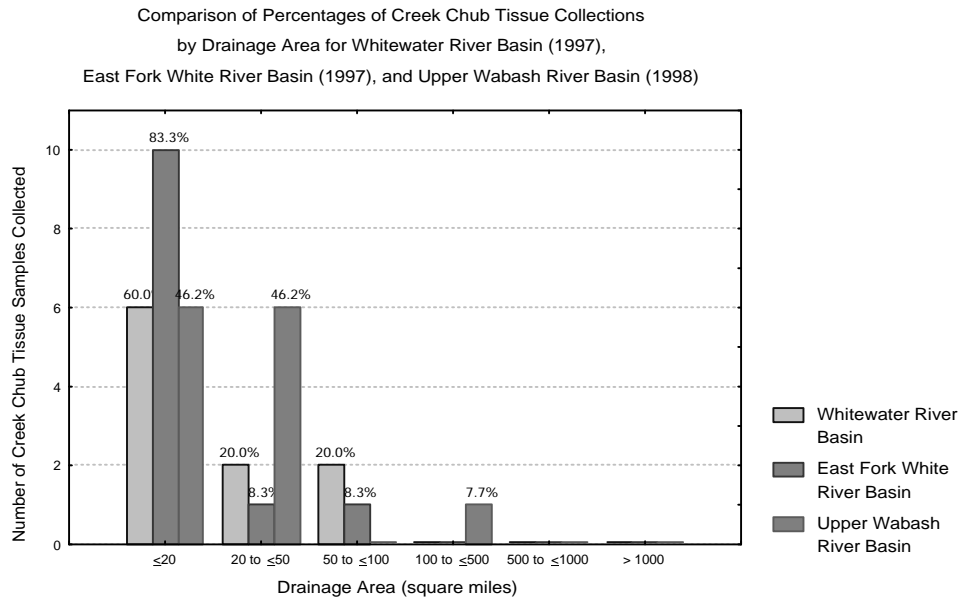


Figure 3. The proportion of creek chub tissue samples collected from stratified probabilistic fish community assessment sites by drainage area for Whitewater River Basin (1997), East Fork White River Basin (1997), and Upper Wabash River Basin (1998).

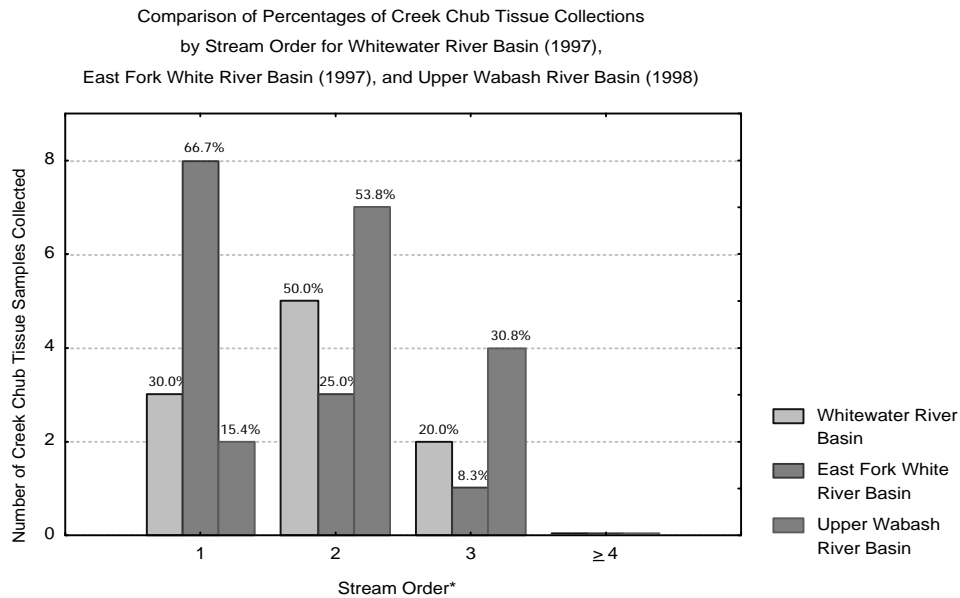


Figure 4. The proportion of creek chub tissue samples collected from stratified probabilistic fish community assessment sites by stream order (Strahler 1957) for Whitewater River Basin (1997), East Fork White River Basin (1997), and Upper Wabash River Basin (1998).

Sixty, 83.3, and 46.2 percent of creek chub tissue samples came from streams of less than twenty square miles of drainage area for the WWR, EFWR, and upper Wabash respectively. Sixty three percent of all creek chub tissue samples collected in 1997 and 1998 came from stream reaches with drainage areas ≤ 20 square miles. A majority of all creek chub tissue samples (88.6%) came from streams of ≤ 50 square miles drainage area. All creek chub tissue samples from probabilistic fish community assessment sites were from streams of third order or less.

For all of the basins, the observed proportions of fish community sites sampled to stream order did not deviate significantly ($p \leq 0.05$) (Chi-square goodness of fit test: EFWR $0.05 < p < 0.10$, WWR $0.90 < p < 0.95$, upper Wabash $0.50 < p < 0.75$) from the expected equal proportions. This means that all fish community samples represent all stream orders equally; thus, the samples are representative of the entire watershed. Creek chub tissue sample collection success did not deviate significantly ($0.10 < p < 0.25$) from the expected proportion of sites per stream order in the WWR Basin. However, creek chub tissue sample collection success did deviate significantly from the expected equal proportions in the EFWR ($0.005 < p < 0.01$) and in the upper Wabash River Basin ($0.025 < p < 0.05$).

A possible explanation for the significant deviations for these two watersheds is that the total number of creek chub tissue samples in each individual basin was rather small. Fourth and greater order streams were then eliminated from consideration and goodness of fit was recalculated. Creek chub tissue sample collections from the EFWR Basin continued to show a significant difference ($0.025 < p < 0.05$) from expected proportional distributions of first, second, and third order streams. By pooling all three basins and eliminating fourth order streams from the goodness of fit test there was no significant deviation ($0.10 < p < 0.25$) from the expected proportions of samples coming from the first, second, and third order streams. By eliminating third order streams from the goodness of fit calculation, the probability increased dramatically ($0.90 < p < 0.95$) that no significant deviation occurred between actual creek chub tissue samples collected and expected proportions of samples coming from first and second order streams.

The mean length for creek chub samples was 14.3 cm ($n=12$) in the EFWR Basin, 14.5 cm ($n=10$) in the WWR Basin, 15.0 cm ($n=13$) in the upper Wabash River Basin, 14.4 cm ($N=22$) for the EFWR and WWR basins pooled together, 14.6 cm ($n=35$) for the 97/98 pooled data set, and 15.5 cm ($n=25$) for the historical targeted sites (Figure 5). A two way t-test for independent sample groups showed no statistical difference ($p > 0.05$) between either the EFWR and the WWR mean lengths, between the pooled 1997 and upper Wabash River Basin (1998) mean lengths, or between the pooled 1997, 1998, 1997 and 1998 pooled, and the historical target site data set ($p > 0.05$). Mean weights were also compared for these data sets (Figure 6). As with the mean lengths, there was no statistical difference ($p > 0.05$) between all of the data groups. The 1997 pooled data had the smallest mean weight but was not significantly different ($p > 0.05$) than either the upper Wabash River Basin (1998), or the historical target sites.

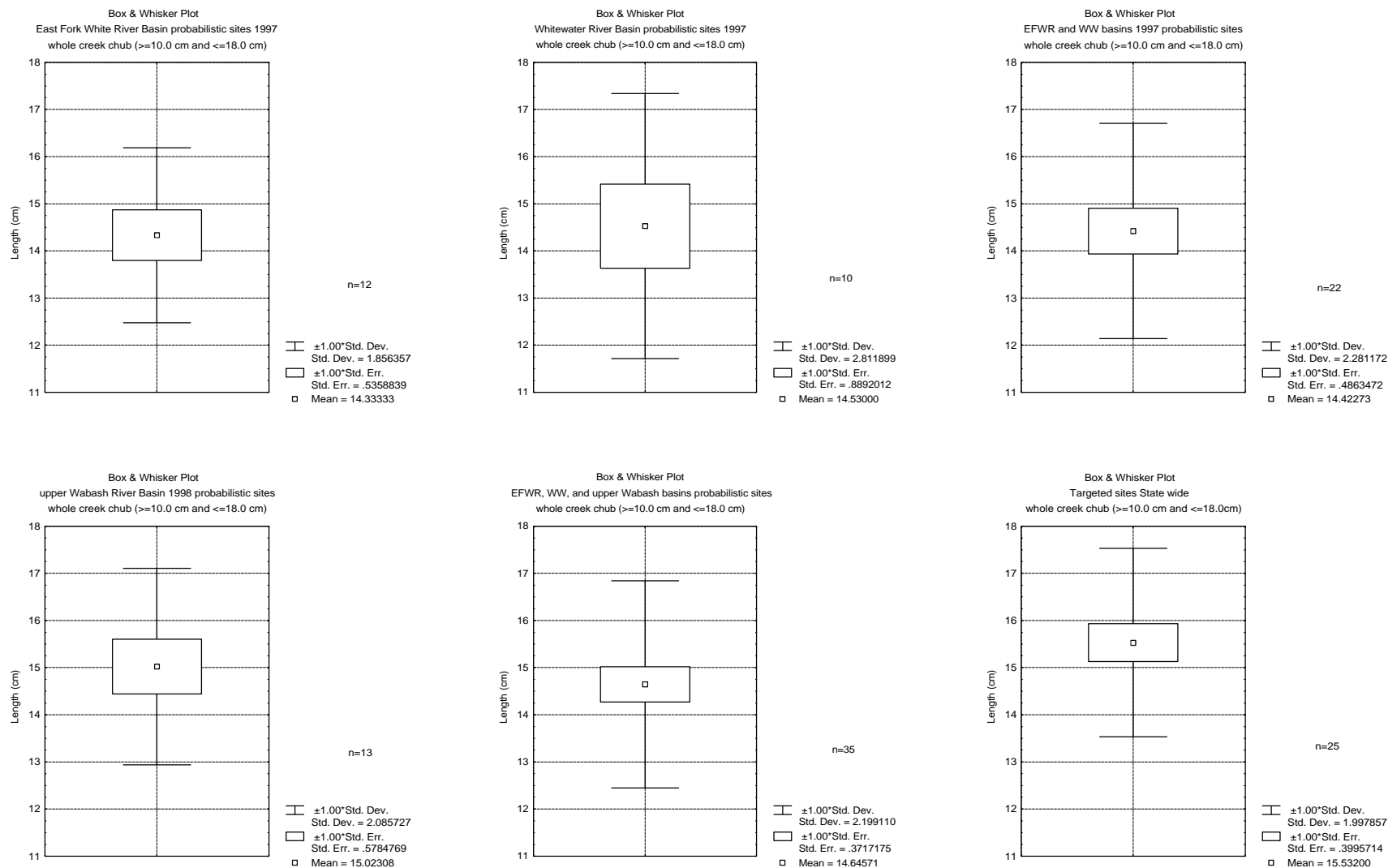


Figure 5. Mean total length (cm) for creek chub samples collected for whole fish tissue contaminant analysis from stratified probabilistic design sites in 1997 and 1998, and for samples collected from historical target sites. Only samples between 10 and 18 cm were included.

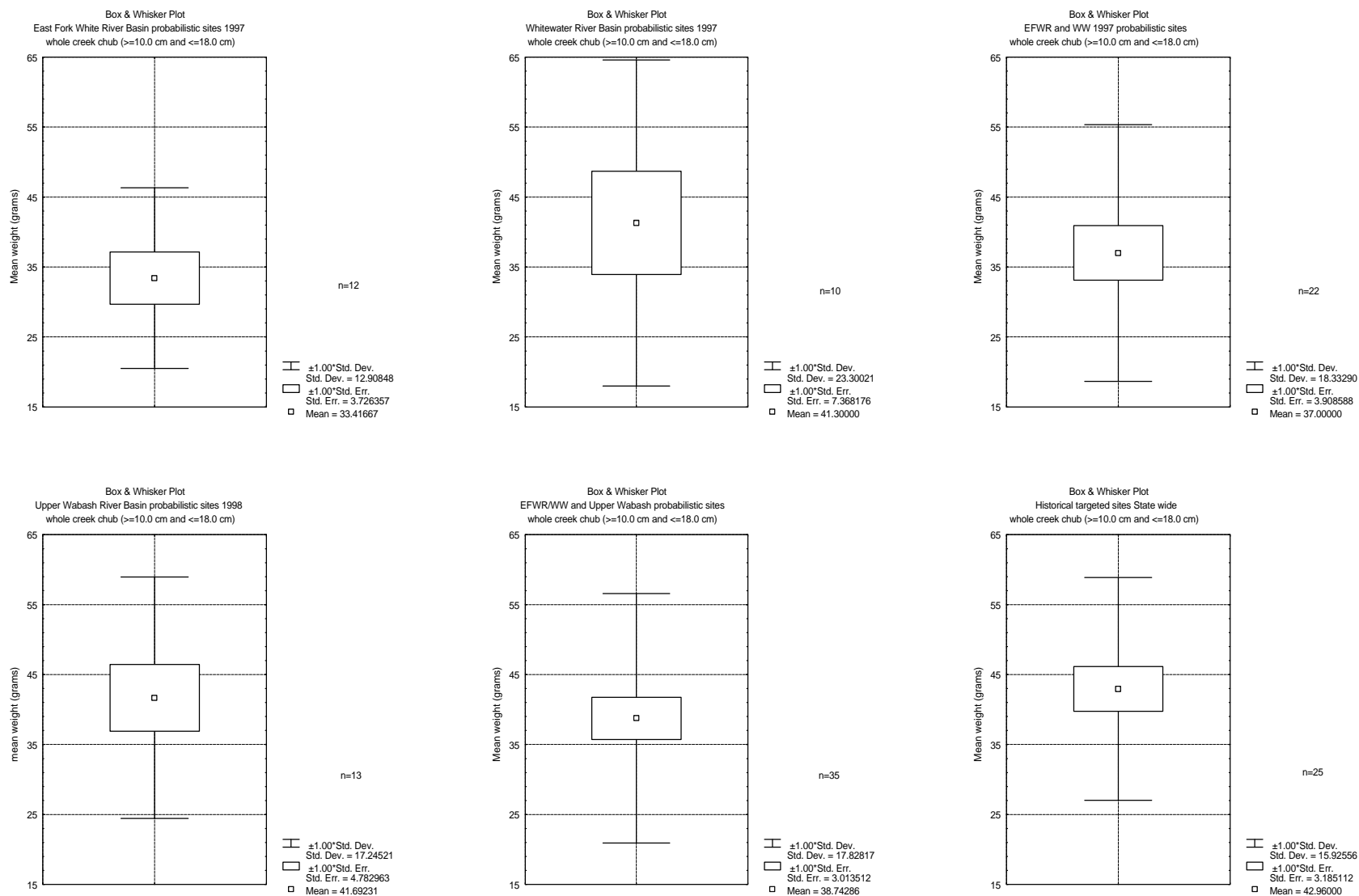


Figure 6. Mean weight (grams) of creek chub samples collected for whole fish tissue contaminant analysis from stratified probabilistic design sites in 1997 and 1998, and for samples collected from historical target sites. Only samples between 10 and 18 cm were included.

Figure 7 shows the mean, standard deviation and standard error of the respective data sets (EFWR, WWR, pooled 1997 data, upper Wabash River Basin (1998), 97/98 pooled, and the historical target sites) for the Log10 mercury concentration (ppb) in the creek chub tissue samples. The data were log10 normalized to reduce the coefficient of variation from >118% to 36% in the 97/98 pooled data set, and from 52 to 13% in the historical target data set. There was no significant difference ($p>0.05$) between the pooled 1997 and the upper Wabash River Basin mercury concentrations in creek chub tissue (Figure 7). There was a significant difference ($p<0.05$) between the probabilistic site creek chub tissue mercury concentrations (both individual basins and pooled data) and the historical target sites data set. The Log10 mean mercury concentration for the 97/98 pooled creek chub tissue samples was 1.36 (23.3 parts per billion (ppb)) while the Log10 mean value for creek chub tissue samples from the historical target sites was 1.82 (65.4 ppb).

There was no significant correlation of mercury concentrations with increasing mean length of whole creek chub composite tissue samples for probabilistic sites and historical target sites (Figures 8 and 9). There was also no significant correlation of mercury concentrations with increasing mean weight of whole creek chub composite tissue samples (Figures 10 and 11). These two factors do not explain the significant differences between the mean concentration of mercury from probabilistic sites and from historical target sites. It is however an indication of the suitability of the selected total length range used for trend assessment purposes with this fish species.

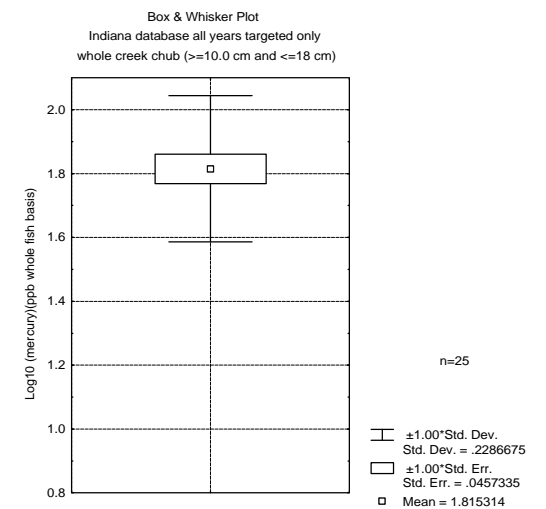
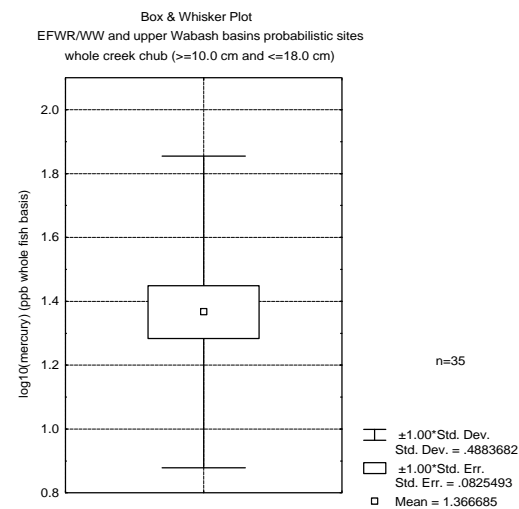
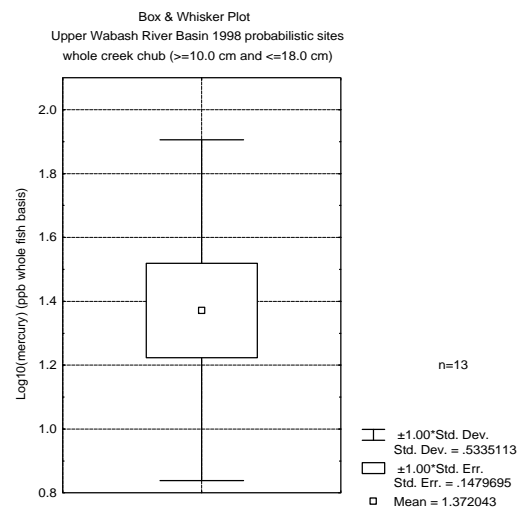
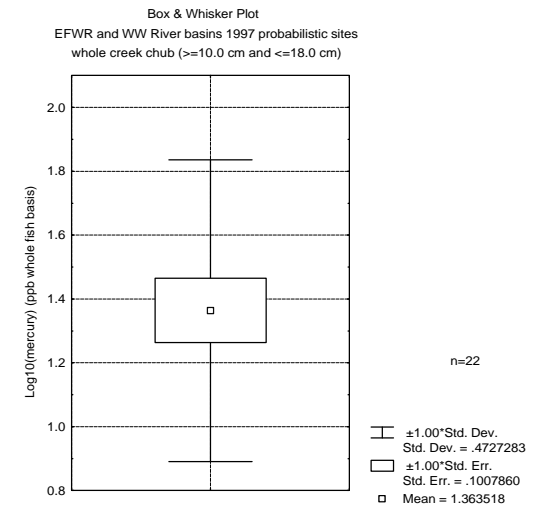
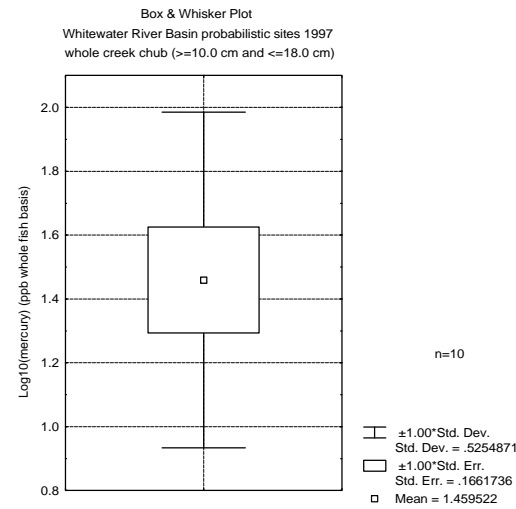
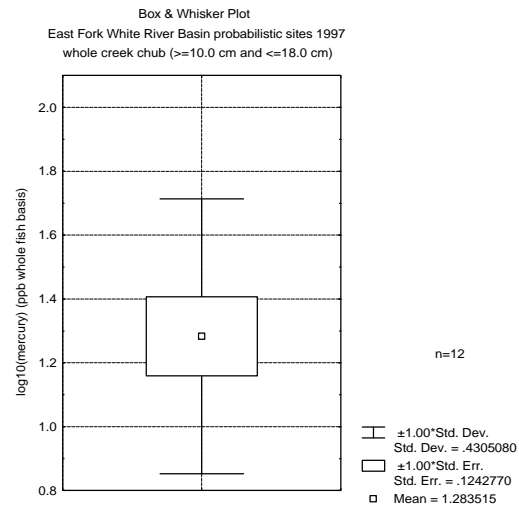


Figure 7. Log 10 mercury concentration (ppb) in creek chub samples collected for whole fish tissue contaminant analysis from stratified probabilistic design sites in 1997 and 1998, and for samples collected from historical target sites. Only samples between 10 and 18 cm were included.

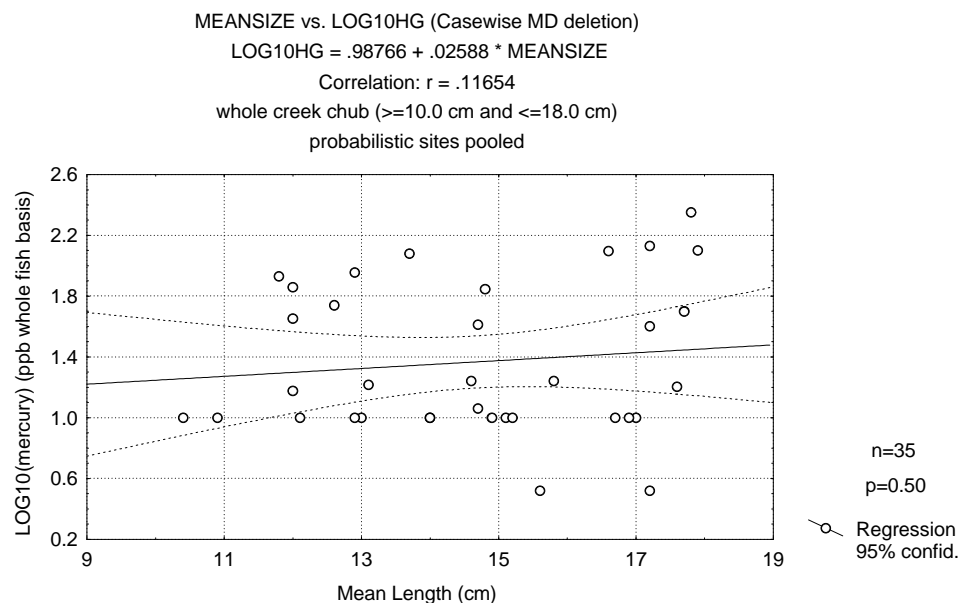


Figure 8. Correlation of mean length (cm) of whole creek chub composite samples to log10 mercury concentrations (ppb) in tissue samples collected from stratified probabilistic fish community assessment sites from Whitewater River Basin (1997), East Fork White River Basin (1997), and Upper Wabash River Basin (1998) pooled.

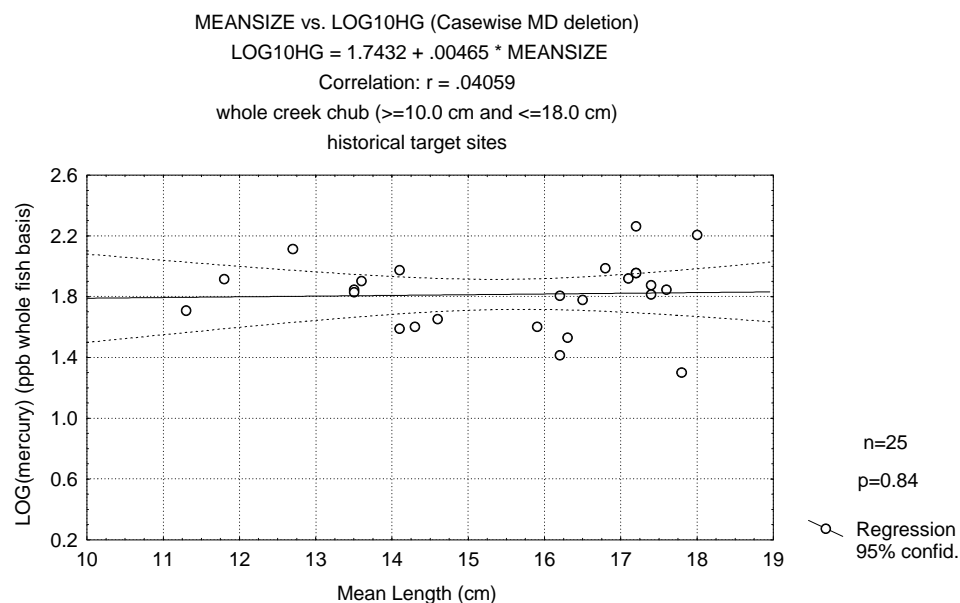


Figure 9. Correlation of mean length (cm) of whole creek chub composite samples to log10 mercury concentrations (ppb) in tissue samples collected from historical target sites.

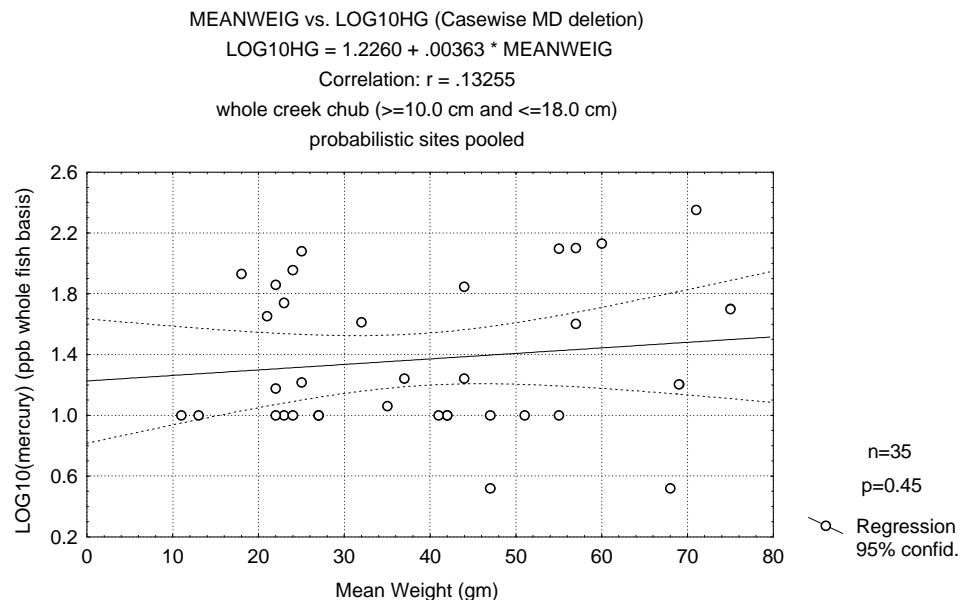


Figure 10. Correlation of mean weight (grams) of whole creek chub composite samples to log10 mercury concentrations (ppb) in tissue samples collected from stratified probabilistic fish community assessment sites from Whitewater River Basin (1997), East Fork White River Basin (1997), and Upper Wabash River Basin (1998) pooled.

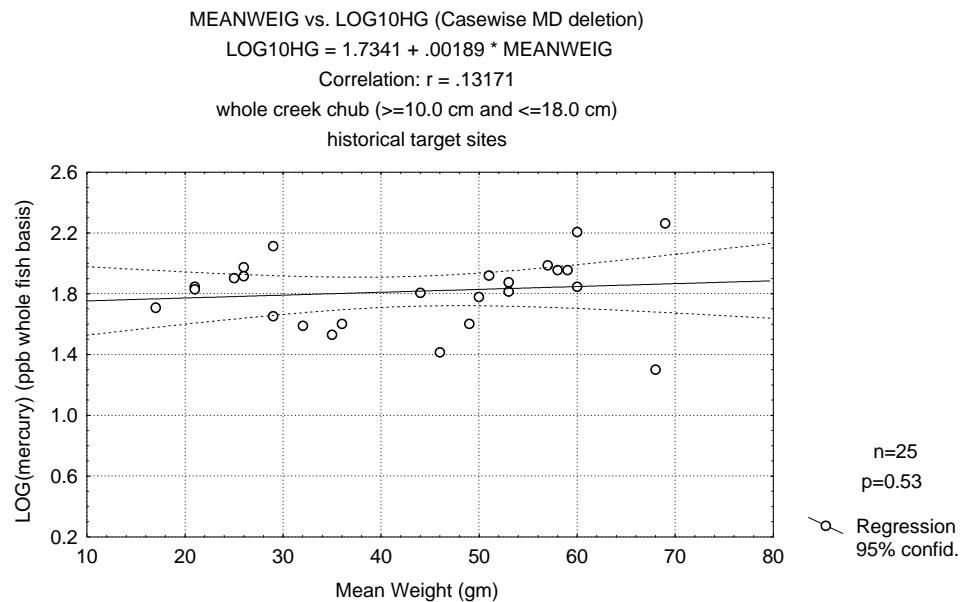


Figure 11. Correlation of mean weight (grams) of whole creek chub composite samples to log10 mercury concentrations (ppb) in tissue samples collected from historical target sites.

DISCUSSION

The mercury concentrations in whole creek chub tissue, using a stratified probabilistic site selection design, is significantly different from mercury concentrations in whole creek chub taken from historical target sites. Within the 10 to 18 cm target size class for mean length, neither changes in size nor weights explain this difference. Because of the weighting for all stream reaches to have an equal probability of being sampled, it was possible to sample extreme stream segments for creek chub populations; therefore, estimations of the true variance for mercury concentrations in creek chub tissue could occur. The target sites tend to be purposely located near, or downstream, of urban centers, specific discharge points, problem areas, bridges, etc., thus biasing the overall estimate of contaminant levels regionally using a sentinel species. There was no difference in mean concentrations of mercury in creek chub tissue samples between the EFWR Basin (1997), WWR Basin (1997), and the upper Wabash River Basin (1998). These are three distinct regions of the State. The significance of this is that if atmospheric mercury deposition is the most influential source of mercury to the aquatic environment, it may be uniformly distributed across the State. The hypothesis will be tested more fully as a complete cycle of fish tissue sample collections from probabilistic sites are completed and assessments are made for the state.

Whole creek chub fish tissue mercury results from the EFWR Basin (1997), the WWR Basins(1997), and the upper Wabash River Basin (1998) probabilistic sites were combined and the 95% confidence interval was calculated. The confidence interval is calculated as the probability of the true population mean (μ) being included between the two values of the interval (Zar 1974). The sample mean is only an estimate of the true population mean, and the calculation of the confidence interval for the population mean (μ) expresses the precision of the estimate. Obviously the tighter the range between the lower and upper limit of the interval, the more accurate the estimate is of the true mean concentration of mercury in the sentinel species (creek chub). Table 1 lists the calculated 95% confidence interval for estimating the population mean of 10 to 18 cm whole creek chub tissue mercury concentrations. It is the establishment of this benchmark measurement that becomes the measure of successes or failures, through time, in our efforts to abate anthropogenic inputs of mercury into the aquatic ecosystem.

Since the collection of fish tissue is secondary to other efforts of data gathering at the probabilistic sites (i.e. surface water chemistry, habitat characterization, macroinvertebrate community assessment, and fish community assessment), it becomes important to understand the minimum sample size needed in order to estimate a population mean (μ) with a desired level of confidence. At issue is the minimization of possible type I or type II errors. Obviously increasing sample size will decrease either possibility. From the results of the 1997 and 1998 combined sampling efforts to collect a creek chub tissue sample from the probabilistic design sites, a minimum sample size needed was calculated for varying levels of confidence or acceptable levels of uncertainty of the mean (Table 2).

Data set	n	coef of var. %	lower limit ug/kg	est. mean ug/kg	upper limit ug/kg
EFWR	12	34	10.2	19.1	36.1
WWR	10	36	12.1	28.8	68.5
1997 pooled	22	35	14.3	23.1	37.4
1998	13	39	11.2	23.6	49.5
97/98 pooled	35	36	15.8	23.3	34.2

Table 1. The 95% Confidence intervals for estimating the population mean of mercury concentrations (**ug/kg**) in 10 to 18 cm whole creek chub from probabilistic design sites. The 1997 pooled data set is of the East Fork White River/Whitewater River basins, and the 1998 data set is of the upper Wabash River Basin.

Level of Confidence	Minimum Number Of Samples Needed
95%	51
94%	35
93%	26
92%	20
91%	16
90%	13

Table 2. Calculation of minimum sample size needed to attain a desired level of confidence of estimating the population mean of whole creek chub fish tissue concentrations of mercury. Based on pooled 97/98 data sets (n=35).

To make regional inferences using creek chub, it is important to understand the distribution of the tissue samples in relation to the original stratification design. The collection of creek chub tissue samples was heavily weighted to the streams of smaller drainage area. Streams of >100 square mile drainage area tended to be under represented for creek chub tissue samples in all three basins. This can be explained by the occurrence of creek chub in mostly smaller order streams for fish community assessment sites (Figure 12). Inferences based on the levels of mercury in creek chub would apply, therefore, to the smaller order streams. However, these smaller order streams make up the majority of river miles and drainage in the basins. First, second and third order streams together may, on average, make up as much as 90% of the stream miles in our three watersheds sampled (U.S. EPA NHEERL, Western Ecology Division). The levels of mercury found in creek chub tissue may be a good relative indicator of basinwide mercury levels and therefore a measure of the risks to fish eating wildlife. Smaller order streams are less subject to point sources of pollution and therefore may be better measures of nonpoint influences such as atmospheric wet deposition.

Comparisons were made of critical wildlife risk values from the Great Lakes Water Quality Initiative (GLWQI) (U.S. EPA 1993) to the cumulative distribution frequency of mercury levels in creek chub from the three watersheds (Figure 13). Whole creek chub (10-18 cm) tissue samples exceeding the critical mercury value for risk to mammalian wildlife will be collected fourteen percent of the time, and forty percent of the time for avian wildlife. Because of the proportional sampling of creek chub tissue samples from only first, second, and third order streams, regional inferences from contaminant results can only be made on these streams; therefore, critical wildlife risk value exceedance proportions can only be applied to river miles of these stream orders.

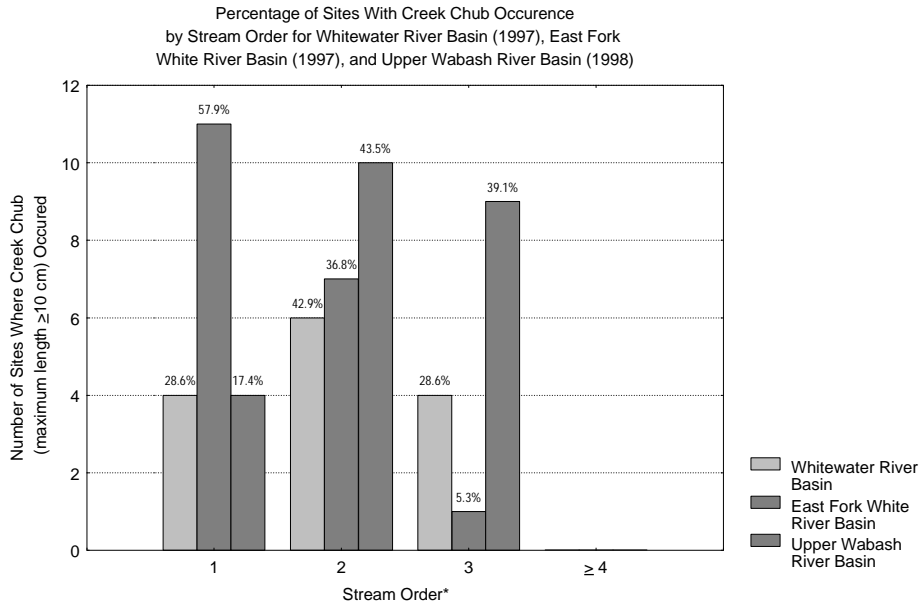


Figure 12. The proportion of creek chub (10-18cm) occurrence at fish community assessment sites by stream order (Strahler 1957) for Whitewater River Basin (1997), East Fork White River Basin (1997), and Upper Wabash River Basin (1998).

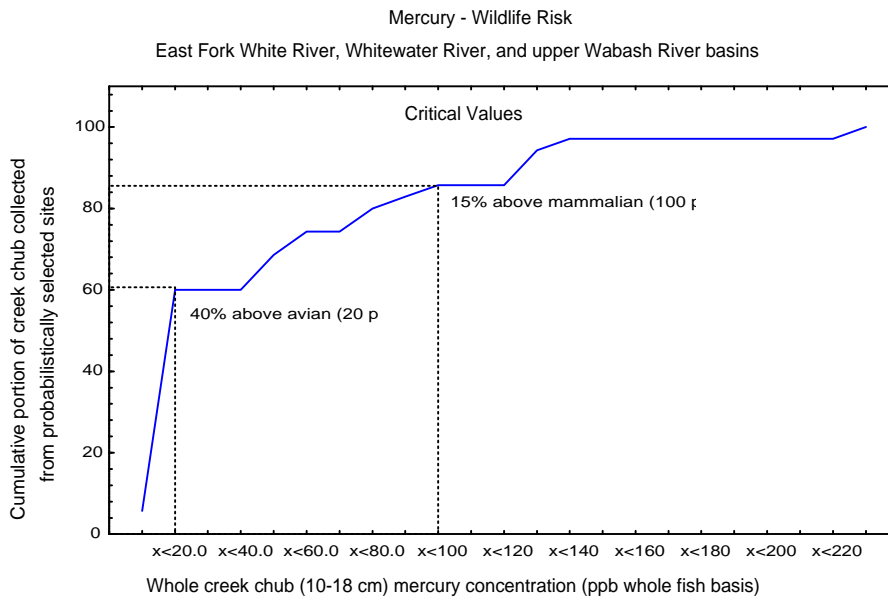


Figure 13. Creek chub (10-18 cm) critical values exceedance for increased risk to wildlife from consumption.

SUMMARY

The Assessment Branch in the Office of Water Management has implemented the management framework set forth in the revised Surface Water Quality Monitoring Strategy and its associated time line in order to establish a scientifically defensible foundation of information from which management decisions can be made (IDEM 1998b). To this end, the Biological Studies Section is in the process of assessing and developing ecological indicator methodologies for aquatic communities and bioaccumulating contaminants. In 1997 the collection of fish tissue for contaminants analyses was added to the Watershed monitoring program of the Surface Water Quality Monitoring Strategy. In this program sites are selected based on a stratified probabilistic design with equal weighting to first, second, third, and fourth or greater stream orders so that inferences can be made on all river miles at a regional scale. The advantage of a probability based design is that it gives estimation of the condition of the entire resource from a limited and manageable number of samples.

The purpose of this report was to evaluate results, to date, of mercury contamination in creek chub tissue samples collected from the probabilistic sites for developing a regional estimation of the mean concentration of mercury in this species as well as estimating the proportion of creek chub exceeding a critical value for risk to fish eating wildlife. The sampling regions assessed were the East Fork White River Basin, the Whitewater River Basin, and the upper Wabash River Basin.

Mean mercury concentration in creek chub tissue from probabilistic sites were significantly lower than a historical data set of tissue samples collected from target sites. In addition, there was no difference in mean mercury concentrations between basins. Whole creek chub tissue mercury results were pooled to calculate a mean mercury concentration of $23.3 \pm \text{ug/kg}$ wet weight basis. By giving an equal probability of all stream reaches being sampled, the probabilistic site based sampling gives a better estimation of the regional mean concentration of mercury in a sentinel species. This becomes a benchmark for measuring successes or failures in abating mercury pollution in our rivers and streams. A minimum of twenty whole creek chub tissue samples would be needed to attain a 92% level of confidence of estimating the regional mean mercury concentration for the creek chub population (10-18 cm).

Sampling success for creek chub tissue was confined to first, second, and third order streams with a fairly equal proportion of samples coming from each. Basinwide inferences based on creek chub tissue mercury contamination levels should be confined to these stream orders. However, first, second, and third order streams combined make up, on average, 90% of the stream miles in the EFWR, WWR, and upper Wabash basins. From this we can infer that as much as 40% of creek chub from these stream orders, in our three basins, may have mercury levels that could pose risk to avian fish eating wildlife, and 15% to mammalian fish eating wildlife. Forty percent of river miles of first, second, and third order streams may contain creek chub with tissue mercury concentrations that could pose a risk to fish eating avian wildlife.

The employment of proper experimental design is critical to properly address questions and issues concerning the extent of environmental contamination. Employing a probabilistic design to site selection for environmental monitoring enables environmental scientists to objectively estimate the extent and variations in the levels of contaminants such as mercury on a regional scale. An objective unbiased approach sets the baseline for monitoring trends in assessing the successes or failures of pollution abatement, and contaminant reduction programs.

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